5

Evoked Potentials in the Visual Pathway of *Heliconius erato*. (Lepidoptera) [1, 2]

S. L Swihart

Department of Biology,
New York State University,
Fredonia, New York

(Plates I-III; Text-figure 1)

[This paper is one of a series emanating from the William Beebe Tropical Research Station of the New York Zoological Society, at Simla, Arima Valley, Trinidad, West Indies. The station was founded in 1950 by the Zoological Society's Department of Tropical Research, under Dr. Beebe's direction. It comprises 250 acres in the middle of the Northern Range, which includes large stretches of government forest reserves. The altitude of the research area is 500 to 1,800 feet, and the annual rainfall is more than 100 inches.

[For further ecological details of meteorology and biotic zones, see "Introduction to the Ecology of the Arima Valley, Trinidad, B.W.I.," by William Beebe, Zoologica, 1952, 37 (13) 157-184.

[The success of the present study is in large measure due to the cooperation of the staff at Simla, especially of Jocelyn Crane, Director, who contributed so freely of her knowledge of the organisms studied. The author particularly wishes to acknowledge the invaluable technical assistance rendered by Mr. Robert Varnum].

Introduction

It is axiomatic that reactive mechanisms are dependent upon sensory perception. A logical extension of this premise is that the nature of behavioral releasers is limited by the action spectra of the sense organs. A not so logical conclusion is that the action spectra of sense organs, and behavioral releasers, are identical. In some cases this may be true; particularly in those situations where a simple sense organ can resolve only the intensity of the stimulus, rather than its "quality." Notable exceptions seem possible in the case of chemoreceptors (Schneider, 1962) and photoreceptors.

While chemical releasers seem to be of primary importance in nocturnal insects, visual stimuli dominate the sensory input of most diurnal species. Behavioral observations of butterflies have confirmed this viewpoint. As Ford (1945) stated, "being day-flying species, the male relies more on sight in finding his mate, though scent may play a small part . . ."

Electrophysiological investigations into the spectral sensitivity of the insect compound eye have often produced luminosity curves, based upon the electroretinogram (ERG), showing a peak in the blue-green (*e.g.*, Autrum & Stumpf, 1953; Goldsmith, 1960). There are, however, instances when behavioral responses seem to be specific for stimuli in other portions of the spectrum. One such case is the neotropical butterfly *Heliconius erato hydara* Hewitson (1869) (see Kaye, 1921), which has been shown to respond preferentially to orange-red stimuli in its feeding and courtship behavior (Crane, 1955). Swihart (1963) demonstrated that the eye's sensitivity, as determined by the electroretinogram, peaked in the blue-green. Use of criteria other than the usual ERG B wave indicated the presence of receptors maximally sensitive to red. A more detailed analysis (Swihart, 1964) gave further indirect evidence for a short latency receptor maximally sensitive to the blue-green (528 mμ), and a long latency one peaking in the red (616–636 mμ).

On the basis of this evidence, it was decided to attempt to follow the passage of information

[1]Supported by a grant (NSF-GB-2331) from the National Science Foundation.

[2]Contribution No. 1,069, Department of Tropical Research, New York Zoological Society.

from the photoreceptors to the brain, in an effort to determine at what level a special sensitivity to the color red develops.

Methods and Materials

Equipment for stimulating photically was as previously described (Swihart, 1963, 1964). This consisted, basically, of an incandescent lamp, narrow-band interference filters, a compur shutter, rotating notched wheel (to provide flicker) and an optical system which focused a spot of light 2–3 mm. in diameter on the cornea of the eye. Stimulus energies were determined by "grease-spot" photometry using a standard light source.

Potentials were amplified with Grass P-6 D.C. amplifiers, displayed on a Tektronix 564, four-beam storage oscilloscope, and recorded with a Grass C-4 camera. In all recordings an upward deflection indicates a negative polarity of the active electrode.

ERGs were recorded with the aid of steel electrodes with a tip diameter of ca. 15 μ placed sub-corneally. Other potentials were recorded with KCl filled, glass capillary electrodes, with tip diameters of 1.5 μ to ca. 8 μ.

Since this investigation involved the establishment of the form and relationship of the potentials characteristic of each of the tissues of the visual pathway, it was arbitrarily decided to compare all responses to a simultaneously recorded ERG. Certain previous efforts in this area (*e.g.*, Burtt & Catton, 1956) had recorded only potentials resulting from various electrode positions. In view of the high degree of variability found in insect visual responses, and their sensitivity to injury, this technique seemed inadvisable, since without continuous monitoring of the ERG one cannot be certain that response characteristics are maintained.

A second advantage of this technique is that it affords a simple method for ascertaining the relative spectral sensitivity of a response by comparison with the ERG. Thus stimuli of two different wavelengths may be adjusted in intensity so as to produce ERGs of identical size. If the simultaneously recorded nervous responses maintain a constant magnitude, then one may presume that the spectral sensitivities of the ERG and the particular nervous response are probably identical. If, however, the ERGs are of the same magnitude, but the nervous responses are significantly different in size, it becomes likely that the nervous response represents the activity of either receptors, or an integrative process with a spectral sensitivity markedly different than those processes responsible for producing the ERG.

Several techniques were tried in determining micro-electrode position. The most satisfactory was found to be inserting the glass electrode with a micro-manipulator equipped with a micrometer scale. As the electrode was passed through the head of the intact insect, the depth of penetration was noted. When the "transit" was completed, the electrode was broken off in situ and the head dissected under a 70× microscope with ocular micrometer. Such a dissection could easily determine the tissues through which the electrode had passed. This could then be correlated with measurements from the micro-manipulator to establish the position of the electrode tip at the time of any given recording.

It should be pointed out that such experiments were conducted with the electrode penetrating the head from every practicable direction. In many cases a dorso-ventral penetration would pass through only a single nervous layer, hence yielding positive information concerning the nature of the response of that tissue. Additional substantiation for electrode location was often found in the fact that nervous responses would remain essentially identical throughout a wide range of electrode penetration, and then suddenly change with a very small shift in electrode position. Dissections confirmed that these changes in waveform corresponded with measurements indicating the simultaneous passage of the electrode into another tissue layer.

Results

Since the purpose of this investigation was to follow the transmission of information from photoreceptors to photocerebrum, the most logical organization of results would seem to be morphological, commencing with the eye itself and progressing proximally towards the brain.

The Eye

Potentials recorded from the most distal portions of the visual pathway (*i.e.*, from the surface of the cornea, or subcorneally) have been the subject of many investigations. The waveform and diurnal variation of the ERG of *H. erato* has been previously published (Swihart, 1963, 1964). Unfortunately, many such studies have utilized such long duration stimuli that the fine structure of the ERG was not apparent.

The most significant small potential is usually the earliest observable response to stimulation. Previously (Swihart, 1963), the day-phase ERG had been reported as possessing an A wave, particularly in response to long wavelength stimulation, and the rising slope of the B wave as containing slight irregularities. This is, indeed, an oversimplification of the complex pattern of interactions which produce the first portion of the ERG. The very earliest observable response

is usually a small, brief, negative potential, with a latency of about 8 msec. If both this potential and an A wave are present, then the negative potential will always precede. Characterized by a short latency and a phasic nature, this potential seems to be little affected by stimulus duration. Fig. 1 illustrates this initial response in ERGs produced by white light stimulation.

At times it is difficult to observe this potential closely. This is due, in large measure, to its being submerged in background noise of nervous origin. The most convenient technique for removing this interference was found to be the suppression of spike potentials by administering a drop of 10% procaine to the preparation. While this markedly affected the ERG waveform, by eliminating the efferent components, the shape of the initial potential did not appear to be modified, and higher amplifications were possible. Figs. 1 & 2 illustrate this potential in anesthetized preparations. Its variation as a function of stimulus wavelength is demonstrated in Fig. 2.

It will be noted that a positive A wave is present in the responses to the longer wavelengths. At shorter wavelengths, the magnitude of this negative potential increases and the latency of subsequent portions of the ERG decreases, thus concealing any positive A wave that might have occurred with a latency comparable to that observed at longer wavelengths. It should be noted that these brief, initial negative responses can normally be recorded only from the vicinity of the cornea. Electrodes placed very much deeper than the distal pigment concentration usually fail to record this phenomenon.

Directly beneath this area large negative responses develop. These appear to be graded action potentials, which develop when the initial response reaches a critical magnitude (Figs. 1 & 3), hence the latency is proportional to stimulus intensity. It is likely that these potentials constitute the largest portion of the leading edge of the ERG B wave (Fig. 4).

When a capillary electrode is placed near the center of the retinula cells (*i.e.,* between the pigment concentrations), simple monophasic negative potentials are recorded. These may be of very large size (up to 10 mV), and seem to be particularly responsive to stimulation with longer wavelengths (Fig. 5.)

One might be tempted to consider this potential as functionally related to those recorded more distally. This interpretation, however, seems most unlikely since these responses have distinctly different spectral sensitivities, with the initial responses being blue-green sensitive, and the deep negative potential being maximally sensitive to red (616 mμ). Latency considerations also tend to substantiate this viewpoint, for the latency of this deep response is usually much greater (*ca.* 15 msec.) than the more distal responses. Recordings from an intermediate location illustrate the independence of this effect and the brief initial negative response (Fig. 6). It appears, therefore, that both this deep, large negative response and the initial phasic response may be considered as receptor potentials, reflecting the activity of two different categories of receptors with different spectral sensitivities.

Still deeper electrode penetration results in producing an entirely different type of response, which is a comparatively short latency positive potential (Fig. 7). Both the spectral sensitivity and latency of this effect seem to be identical to that of the deeper, large negative potential. It is sometimes difficult to obtain good recordings of this waveform in an intact preparation due to its extreme localization. It is, however, much simpler in the case of a procaine-anesthetized animal, under which circumstances it invades and dominates the responses recorded from much of the nervous tissue in the visual pathway.

It should be noted that this potential is most clearly recorded from the region of the proximal pigment cells and in the tracheated tissue layer and not in the lamina gangularis, as has been previously suggested (Autrum, 1958).

On the basis of action spectrum and latency, it is the author's opinion that these positive potentials represent a reflection of the deep negative response, induced by current flow resulting from receptor depolarization, and spreading electrotonically. It seems likely that it is this potential which is responsible for the positive ERG A wave at long wavelengths.

The lamina gangularis

Much discussion has arisen concerning the role of the lamina gangularis. Recordings from this area typically present a waveform consisting of a large, but brief, positive potential, followed by a sustained negativity. It appears that this waveform represents the summation of the previously discussed positive potential, and a graded action potential of negative polarity (Fig. 8). The apparent similarity in the sizes of the positive potential in this figure is somewhat misleading, since vastly different white light stimulus intensities will also produce matching positive deflections. It seems likely that this constancy in size is due to the interaction of two components of opposite sign which vary nearly proportionately and hence maintain a nearly constant relationship. The negative potential recorded at this level clearly has a different spectral sensitivity than the ERG, being maximally sensitive to long wavelengths.

Medulla externa

Responses recorded from this region appear to be largely of spike potential origin. Swihart (1964) described the patterns of single fiber responses observed in this general region of the visual pathway. No additional types of fibers have been observed. During the course of these experiments, slightly larger electrode tip diameters and the use of electrical filters served to assist in the recordings of summated potentials from small areas of the neuropile. Such recordings illustrate that there is a morphological localization of specific fiber types.

Swihart (1964) emphasized the role of efferent components in the ERG. Most significant of these effects were: (1) a B wave nervous component, excitatory in function, resulting from the summated response of "on" fibers, maximally sensitive to the blue-green and probably originating in the medulla interna; (2) a positive "dip" following the B wave, which served to inhibit receptor depolarization, resulting from the inhibition of spontaneously active, red-sensitive, neurons, located in the medulla externa; and (3) an "off" effect (D wave), arising in the medulla interna from the activity of red-sensitive fibers.

Recent investigations have supported the previous observations, with one exception: the origin of the B wave nervous component. Nearly pure "on" responses were most clearly recorded from the proximal periphery of the medulla externa (*i.e.*, the vicinity of the cortex), particularly in those regions not closely covered by the lamina gangularis (Fig. 9).

Recordings from the interior of the externa produce a sustained positive response which appears to be a summated response reflecting the inhibition of spontaneously active neurons (Fig. 10). Latency considerations clearly differentiate between the positive potentials which arise in the receptors (latency *ca.* 15 msec.) and those observed in the medulla externa (latency *ca.* 25 msec.).

It is interesting to note that the B wave nervous component responds to an increase in stimulus intensity by a decrease in magnitude, while the size of the positive response is directly proportional to stimulus intensity (Fig. 11). This is precisely what would be expected if the B wave component were excitatory, and the positive potential inhibitory, with their interaction tending to stabilize the degree of receptor depolarization. Such complex responses to stimulus intensity make it difficult to meaningfully evaluate the spectral response of these effects. It should, however, be noted that the positive response is often preceded by a brief positive potential which can easily be distinguished from the response itself (Fig. 12). It seems likely, therefore, that the level of spontaneous activity is regulated by the electrotonic potentials which originate in the receptors.

Morphologically, the medulla externa resembles a cup-shaped structure, with the opening towards the posterior. Thus, only the distal half of the "cup" is located directly beneath the receptors. In the anterior and proximal portions of the structure, the magnitude of the positive response is considerably reduced. In this region a negative potential, similar to that recorded in the lamina gangularis, becomes dominant (Fig. 13). This description may also be applied to potentials recorded from the internal chiasma.

Medulla interna

The medulla interna is structurally the smallest of the tissues of the visual pathway, being located in the "cup" formed by the medulla externa. Recordings from the posterior surface of this structure reveal a pure "off" response (Fig. 14).

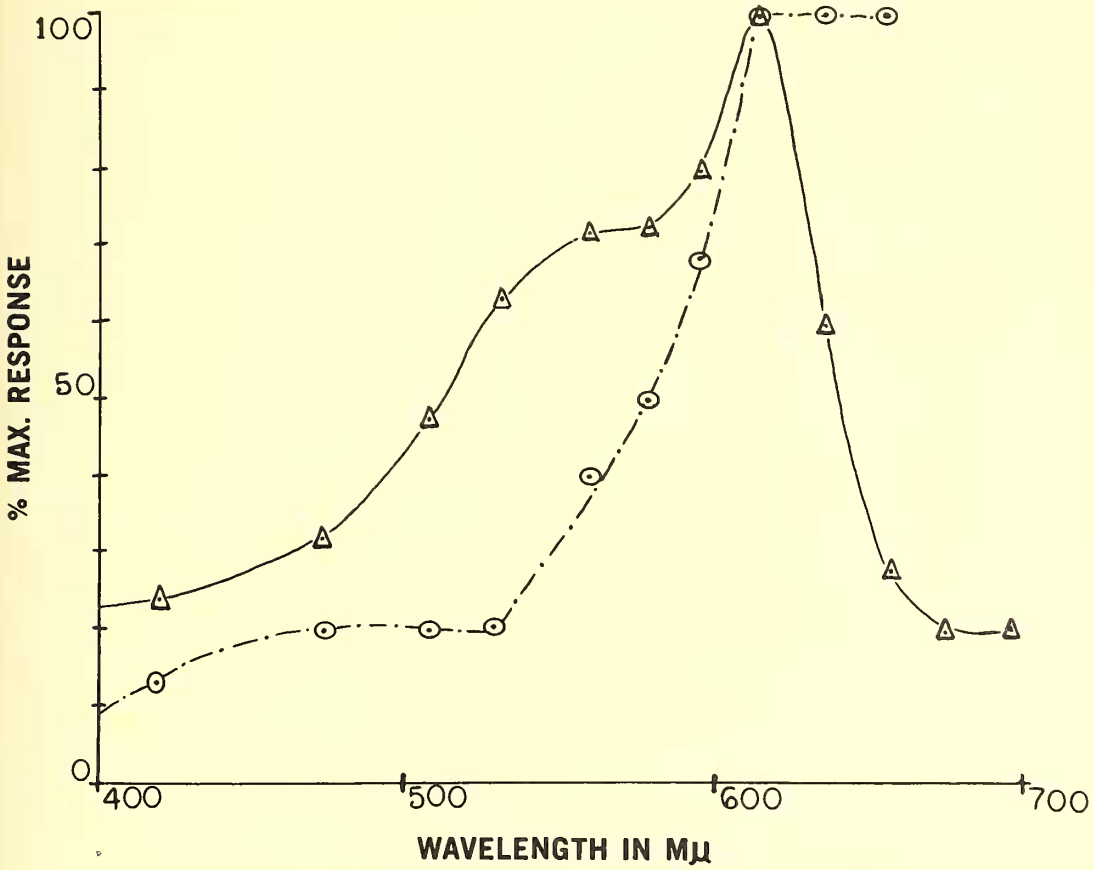
The interior, however, produces a large negative response (Fig. 15) with an extremely long latency (*ca.* 30 msec.), and great sensitivity to long wavelengths (Text-fig. 1). It would seem that this response represents the activity of fibers characterized previously (Swihart, 1964) as showing maximal activity during the C wave.

Such long latencies suggest that such a response would be incapable of following a rapidly flickering stimulus. This is indeed the case; flicker fusion frequencies (F.F.F.) of the various responses vary greatly, being related to the latency of the particular effect. Thus the F.F.F. of the large negative receptor response, recorded from the center of the eye, is markedly lower than that of the ERG (Fig. 16). The frequency reaches a low of less than 40 c.p.s. in certain portions of the brain.

Protocerebrum

Two distinctly different types of response appear to dominate the main (dorsal) mass of the protocerebrum. The posterior portion demonstrates a brief, long latency, positive potential (Fig. 17). which could possibly represent an inhibition of the spontaneous activity known to exist in the mushroom bodies. This response is usually of low magnitude, and difficult to analyze, but appears to be proportional in size to the magnitude of the ERG B wave (*i.e.*, blue-green sensitive).

The anterior, dorsal portion of the protocerebrum shows a negative response which closely resembles that observed in the medulla interna (Fig. 18). In fact, it seems more than likely it is this effect which induces this brain response.



TEXT-FIG. 1. Luminosity curves obtained by ploting the responses recorded from the medulla interna (long latency, negative response, see Fig. 15). The solid line with triangles indicates the magnitude of the responses elicited by stimulation with constant energy (*ca.* 300 microwatts). The plot formed by dashed lines and circles represents the magnitude of the same response, as measured in another individual, when stimuli intensities were adjusted so as to produce equal magnitude ERG B waves. It will be observed that in each case the maximum response is reached at the same wavelength (616 mμ). Note also the manner in which the response is maintained at a constant magnitude, at longer wavelengths, when the ERG magnitude is maintained at a constant size. This would seem to indicate that the receptor responsible for producing the medulla interna response is also responsible for the ERG B wave at wavelengths greater than 616 mμ.

Fig. 19 illustrates the result of single fiber recordings from this region. It will be noted that spike potentials are associated with a sustained negative potential (somewhat suppressed in these recordings due to the use of a high frequency bandpass filter). Since both the magnitude and duration of this potential is greater in response to stimulation with long wavelengths (for a given magnitude of ERG), a greater response in terms of spike potentials is induced by such stimuli. While short wavelength, high intensity stimuli may produce a high instantaneous spike frequency, the longer wavelengths will produce a train of spikes with a duration at least 25% longer (comparison of 528 and 616 mμ). Low intensity, short wavelength stimuli may completely fail to elicit spike potentials, while longer wavelengths produce well defined trains.

Electrodes which penetrate the ventral portions of the protocerebrum record a large variety of response types. Many of these are of a complex waveform, with latencies much shorter than the previously described "brain" responses (Fig. 20). The physical distribution of these potentials is, however, so narrow that they are extremely difficult to work with experimentally.

CONCLUSIONS

These experiments have, in general, confirmed previous observations concerning the origin and nature of those potentials which comprise the ERG. Fig. 21 illustrates the close match in slope

and time between various nervous effects and portions of the ERG.

It has become clear that the conventional terminology for the ERG waveform is completely inadequate. Its adoption seems to have arisen from the convenience of drawing a parallel between the vertebrate ERG and the similarly shaped (but of inverted polarity) insect ERG. This has led to the unfortunate situation whereby the terminology bears little relationship to biological reality. Thus, for example, the term "B wave," rather than designating a single homogenous potential, may well include as many as four distinctly different components (*i.e.,* two receptor potentials, a graded action potential and one of nervous origin).

The following terminology is, therefore, proposed as retaining the advantages of the present system, plus containing the precision necessary for accurate description of insect ERG waveforms.

a (alpha); the initial phasic negative potential of receptor origin.

A; retains its identification with the initial positive deflection which originates deep in the receptors.

B; is maintained as a generic term referring to the complex, large, negative "on" effect, which may contain several components:

B′; a graded action potential,

B″; a large, receptor potential, and

B‴; which is due to the activity of "on" fibers in higher nervous centers.

C; continues as a generic term referring to the response which is maintained throughout the duration of stimulation. The C wave is composed of two components of opposing polarity:

C′; which is of negative polarity, and consists largely of potentials of receptor origin,

C″; is of positive polarity, and is of nervous origin.

D; continues to refer to the ERG "off" effect which also appears to have the possibility of containing two different components.

D′; which is the potential which dominates the wave, and is due to the activity of "off" fibers,

D″; which arises as an overshoot in the recovery of the spontaneously active neurons. It is possible that there are other effects which contribute to the ERG "off" response.

These experiments have been purposefully confined to the day-phase ERG, since its complexity and behavioral associations were most intriguing. Preliminary observations on the night phase response indicate the presence of at least some of the same types of nervous activity, particularly the spontaneous activity of the medulla externa, albeit at a very much reduced level. Elucidation of the exact source of the negative potential which dominates the night-phase ERG awaits further experimentation.

It will also require much additional effort before it becomes possible to state with certainty the complete chain of events which are responsible for the passage of information from the eye to the brain. In many cases complex waveforms were recorded (*e.g.,* Fig. 11) which were analyzed as being a summation of several simpler patterns of response. This assumption may not be entirely true, and other types of activity, not described, may be significant.

In spite of such unanswered problems, certain general conclusions are possible. It is increasingly certain that in the case of *H. erato,* there are two distinct types of receptors, with differing spectral sensitivities, latencies, types of "output" etc. Despite considerable interaction between the receptors, their outputs remain sufficiently independent to permit their association with particular patterns of nervous activity arising at discrete locations along the visual pathway.

Those effects which arise in the medulla externa (*i.e.*, B‴, C″) appear to serve primarily in the regulation of receptor activity, rather than the transmission of information. Although somewhat unlikely, it is possible that the inhibition of spontaneous activity in the externa may also serve to transmit information to the interna, in a manner analogous to that observed in the insect ocellus (Ruck, 1961).

In any case, either directly or indirectly, the red-sensitive receptor appears to be primarily responsible for inducing the large, long latency response found in the medulla interna.This effect is doubtless a key step in the transmission of information to certain centers in the brain. It thus serves to induce an activity pattern in neurons in these higher centers which continues to exhibit a disproportionate sensitivity to the color red.

It seems clear, therefore, that the behavioral sensitivity of *H. erato* to long wavelengths can be given a firm basis in the physiology of the eye, and the existence of specific neuronal pathways that serve to mediate the transmission of sign stimuli.

It is equally clear, however, that the analysis of the sensory input from composite potentials, such as the ERG, is totally unsatisfactory. As an example, Magnus (1956) reported a behavioral F.F.F. of about 75 c.p.s. for the fritillary *Argynnis paphia,* while the ERG demonstrated a F.F.F.

of 151 c.p.s. While this may be partially explained by differences in stimulus intensity (Ferry-Porter law), it seems certain that it is largely due to the limited capability of the nervous system to transmit high F.F.F. Thus *H. erato* shows a maximum F.F.F. subcorneally of of about 160 c.p.s., and a F.F.F. at the medulla interna of about 75 c.p.s.

It is firmly believed that continued electrophysiological investigations into the responses characteristic of the insect higher nervous centers will provide further information concerning the physiological basis of innate behavior patterns.

References

Autrum, H.

1958. Electrophysiological analysis of the visual system in insects. Exp. Cell Res., Suppl., 5: 426-439.

Autrum, H., & H. Stumpf

1953. Electrophysiologische Untersuchungen uber das Farbensehen von *Calliphora*. Zeit. vergl. Physiol., 35: 71-104.

Burtt, E. T., & W. T. Catton

1956. Electrical responses to visual stimulation in the optic lobes of the locust and certain other insects. J. Physiol., 133: 68-88.

Crane, J.

1955. Imaginal behavior of a Trinidad butterfly, *Heliconius erato hydara* Hewitson, with special reference to the social use of color. Zoologica, 40: 167-196.

Ford, E. B.

1945. Butterflies. Collins, London. xiv + 368 pp.

Goldsmith, T. H.

1960. The nature of the retinal action potential and the spectral sensitivities of ultra-violet and green receptor systems of the compound eye of the worker honeybee. J. Gen. Physiol., 43: 755-799.

Kaye, W. J.

1921. A Catalogue of the Trinidad Lepidoptera, Rhopalocera (Butterflies). Mem. Dept. of Agriculture, Trinidad and Tobago, No. 2, xii + 163 pp.

Magnus, D. B.

1956. Experimental analysis of some "overoptimal" sign-stimuli in the mating-behavior of the fritillary butterfly *Argynnis paphia* L. (Lepidoptera: Nymphalidae). Proc. Tenth Inter. Cong. of Ent., 2: 405-418.

Ruck, P.

1961. Electrophysiology of the insect dorsal ocellus. J. Gen. Physiol., 44: 605-657.

Schneider, D.

1962. Electrophysiological investigation of the olfactory specificity of sexual attracting substances in different species of moths. J. Ins. Physiol., 8: 15-30.

Swihart, S. L.

1963. The electroretinogram of *Heliconius erato* (Lepidoptera) and its possible relationship to established behavior patterns. Zoologica, 48: 155-165.

1964. The nature of the electroretinogram of a tropical butterfly. J. Ins. Physiol., 10: 547-562.

EXPLANATION OF THE PLATE

PLATE I

FIG. 1. ERG recorded with steel, subcorneal electrode. Procaine anesthesia. Responses to white light stimulation (*ca.* 62,000 microwatts) of various durations (10, 20 and 40 msec.) superimposed. Upward deflection of lower trace indicates period of stimulation.

FIG. 2. Initial portions of ERG response to various colors, procaine anesthesia, steel electrode. Sweep was triggered 1.5 msec. after onset of stimulation. Stimulus energy about 300 microwatts at each wavelength.

FIG. 3. Simultaneous recording from cornea (top trace) and vicinity of distal pigment cells (latter with capillary electrode), demonstrating the variation in response characteristic of various stimulus intensities; procaine anesthesia. Eight responses are superimposed, each representing a white light stimulus with twice the energy contained in the preceding. Maximum stimulus *ca.* 62,000 microwatts. Time base as in Fig. 2. Stimulus duration, 20 msec. (bottom trace), with delayed sweep trigger. Note that the initial superficial response has a relatively constant latency (about 12 msec. in this specimen), unlike the deeper response.

FIG. 4. Recordings from the same sites as in Fig. 3, except without anesthesia. Demonstrates the superimposed responses to two different colors (blue-green, 528 mμ and red, 616 mμ) with intensities adjusted so as to produce equal magnitude ERG B waves (upper trace). Note that in this and all subsequent ERG recordings, red produces the greatest "dip" following the ERG B wave, and the largest "off" response. Stimulus duration 100 msec. Note also that the receptor responses (center trace) match each other, and that the size, slope and timing of the leading edge of the receptor response are identical to the leading edge of the ERG B wave.

FIG. 5. Comparison of ERGs and response from the center of the retinula cells (between pigment cells). Details of stimuli as in Fig. 4. Note that the response to the longer wavelength is more than twice as great as that elicited by the blue-green, even though similar-sized ERGs are produced. It will also be noted that the size, slope and latency of this response are markedly different from that of the ERG B wave.

FIG. 6. Stimulation and recording as in Fig. 5. except that in this case the capillary electrode picked up part of the initial, superficial response also. This is more clearly shown in the photographic enlargement of the leading edge of the recording of receptor activity (part B). Note that the initial responses match in size and shape when the ERGs match (*c.f.*, Fig. 2 where constant energy stimuli was employed). The latency and magnitude of the deeper response are, however, totally independent of the size of the initial response.